Reference 2

For general laboratory use.
FOR *IN VITRO* USE ONLY.

Pwo DNA Polymerase

From Pyrococcus woesei
Deoxynucleoside-triphospate:
DNA deoxynucleotidyltransferase, EC 2.7.7.7

Cat. No. 1 644 947 100 units
Cat. No. 1 644 955 500 units (2 × 250 units)

Version 3, April 2002
Store at −15 to −25°C

Product description

Volume activity
1–5 × 10^3 units/ml as determined in the assay on activated DNA.

Storage and dilution buffer
20 mM Tris-HCl, pH 7.5 (20°C), 100 mM KCl, 1mM dithiothreitol (DTT), 0.1 mM EDTA, 0.5% Nonidet[1] P40 (v/v), 0.5% Tween[2] 20 (v/v), 50% glycerol (v/v).

Stability
The undiluted enzyme solution is stable when stored at −15 to −25°C at least until the date stated on the label.

Supplied buffers and solution
- **PCR buffer, 10 × conc. with $MgSO_4$:** 100 mM Tris-HCl, pH 8.85 (20°C), 250 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$
- **PCR buffer, 10 × conc. without $MgSO_4$:** 100 mM Tris-HCl, pH 8.85 (20°C), 250 mM KCl, 50 mM $(NH_4)_2SO_4$
- **$MgSO_4$ stock solution:** 25 mM $MgSO_4$

Unit assay on activated DNA
a) Incubation buffer for assay on activated DNA: 20 mM Tris-HCl, pH 8.8 (20°C), 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.2 mM of each dATP, dCTP, dGTP, dTTP.

b) Incubation procedure: 12.5 mg activated (1) calf thymus DNA and 0.1 mCi [α-^{32}P]dCTP are incubated with 0.01–0.1 units Pwo DNA polymerase in 50 µl incubation buffer with a paraffin-oil overlay at 70°C for 30 min. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation followed by scintillation counting.

Unit definition
One unit Pwo DNA polymerase is defined as the amount of enzyme that catalyzes the incorporation of 10 nmol total deoxynucleoside triphosphates into acid precipitable DNA within 30 min at 70°C under the conditions described above.

Properties
Pwo DNA polymerase was originally isolated from the hyperthermophilic archaebacterium Pyrococcus woesei. The enzyme has a molecular weight of about 90 kD. It is a highly processive 5'–3' DNA polymerase and possesses an 3'–5' exonuclease activity also known as proofreading activity. The enzyme has no detectable 5'–3' exonuclease activity.

Pwo DNA polymerase exhibits increased thermal stability with a half life of greater than 2 h at 100°C compared to Taq DNA polymerase with a half life of less than 5 min at this temperature.

The inherent 3'–5' exonuclease proofreading activity of Pwo results in an over 10-fold increased fidelity of DNA synthesis compared to Taq DNA polymerase. Pwo DNA polymerase generated PCR products are blunt-ended and can therefore be used directly for blunt-end ligation without any pretreatment of the ends.

Fidelity of *in vitro* DNA polymerization is one of the most important subjects in PCR. For many applications of PCR, where a homogenous DNA population is analyzed (i.e. direct sequencing or restriction endonuclease digestion), the mutations that are induced by the polymerase during PCR are of little concern. However if only a small amount of template DNA or RNA is used as starting material and if after PCR single DNA molecules are analyzed, PCR artifacts can be a significant problem.

Fidelity of DNA polymerization is for instance important for:

- cloning of PCR products
- study of allelic polymorphism in individual RNA transcripts (1, 2)
- characterisation of the allelic stage of single cells (3) or single DNA molecules (4, 5)
- characterisation of rare mutations in tissue (6)
- characterisation of a population of cells in culture

When using Taq DNA polymerase [error rate of 2 × 10^{-4} errors/base (7)] about 56% of a 200 bp amplificaton product will contain at least a single error after 1 million fold amplification. In contrast when using Pwo DNA polymerase for amplification only 10% of the products will contain an error under the same conditions (error rate of 3.2 × 10^{-6}).

Application protocols

The optimal reaction conditions (incubation times and temperatures, concentration of Pwo DNA polymerase, template DNA, Mg^{2+}-ions) depend on the template/primer pair and must be determined individually. It is especially important to titrate the Mg^{2+}-concentration and the amount of enzyme required per assay. Optimal Mg^{2+} concentrations are in the range of 1–10 mM.

Whereas Taq DNA polymerase requires $MgCl_2$ for optimal activity Pwo shows higher activity with $MgSO_4$. The standard concentration of Mg^{2+} is 2.0 mM for Pwo. Optimal enzyme concentrations range from 0.5–5 units per assay. The standard concentration is 2.5 units.

The dNTPs (e.g. PCR Nucleotide Mix,*) should be added to the incubation mixture directly before use. This will prevent decomposition of deoxynucleoside triphosphates that can occur at the alkaline pH required for optimal enzyme activity. The recommended length of template DNA is 3 kb.

Note
In the absence of dNTPs, the 3'–5' exonuclease activity associated with Pwo will begin to degrade template and primer DNA. Therefore, it is important to always add Pwo DNA polymerase to the reaction mixture last. This can be achieved by using the hot start technique with AmpliWax[3]. In this case primer and template are separated from the polymerase by preparing appropriate upper and lower mixes. Both mixes are separated by a wax layer, that melts above 70°C and allows then thorough mixing of the reagents.

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Tgo DNA Polymerase

From Thermococcus gorgonarius
Deoxynucleoside-triphosphate: *DNA deoxynucleotidyltransferase, EC 2.7.7.7*

Cat. No. 3 186 172 50 reactions
Cat. No. 3 186 202 100 reactions
Cat. No. 3 186 199 250 reactions

Version 1, Jan. 2003

Store at –15 to –25° C

Product overview

Origin

Tgo DNA polymerase is a new enzyme preparation originally isolated from Thermococcus gorgonarius.

Characterization

The supplied enzyme is the recombinant form and purified to be free of unspecific nucleases. The enzyme has a molecular weight of about 90 kD. Tgo DNA polymerase exhibits increased thermal stability with a half-life of more than 2 h at 95 °C compared to Taq DNA polymerase with a half-life of less than 5 min at this temperature.

Proofreading activity

Tgo DNA Polymerase is a highly processive 5´-3´ DNA polymerase and possesses a 3´-5´exonuclease activity, also known as proofreading activity. The enzyme has no detectable 5´-3´ exonuclease activity.

The inherent 3´-5´ exonuclease/proofreading activity of Tgo DNA polymerase results in a superior fidelity of DNA synthesis compared to Taq DNA Polymerase and other commercially available enzymes with proofreading activity.

Comparison of fidelity of different thermostable DNA Polymerases (3)

Enzyme	Error Rate*	Mutation Frequency**
Taq DNA Polymerase	1.3×10^{-5}	5.1 %
Other commercially available proofreading polymerases	1.8×10^{-5} to 8×10^{-7}	5.1 – 0.3 %
Tgo DNA Polymerase	4.9×10^{-7}	0.2 %

* Error rate calculated according to Keohavong and Thilly (4)
**percentage of lacI⁻ colonies

Application

The accuracy or fidelity with which a given DNA fragment is amplified during PCR is of vital importance for certain experiments. For applications of PCR, where a homogenous DNA population is analyzed (i.e. direct sequencing or restriction endonuclease digestion), the mutations induced by the polymerase are of little concern. However if only a small amount of template DNA or RNA is used as starting material and if single DNA molecules are analyzed after PCR, artifacts can be a significant problem.

Fidelity of DNA polymerization is of particular importance for the

- cloning of PCR products
- study of allelic polymorphisms in individual RNA transcripts
- characterization of the allelic stage of single cells or single DNA molecules
- characterization of rare mutations in tissue
- characterization of a population of cells in culture

Volume activity

1 U/µl as determined in the assay on activated DNA.

Storage and dilution buffer

10 mM Tris-HCl, pH 8.5 (4°C), 50 mM KCl, 10 mM 2-Mercaptoethanol, 0.1 mM EDTA, 0.5% Nonidet P40 (v/v), 0.5% Polydocanol (v/v), 50% Glycerol (v/v).

Supplied Tgo Reaction Buffer, 5 x conc. (including $MgCl_2$)

50 mM Tris-HCl, pH 8.5 (25°C), 87.5 mM $(NH_4)_2SO_4$, 6.25 mM $MgCl_2$, 2.5% Tween20 (v/v), 7.5% DMSO (v/v).

Note: Storage of the buffer for a longer time at 2 to 8°C or on ice may result in the precipitation of Mg^{++} ions. Store the buffer at -15 to -25°C, thaw at 37°C and vortex carefully before use.

Enzyme storage/ stability

The undiluted enzyme solution is stable when stored at -15 to -25°C until the expiration date printed on the label.

Unit assay on activated DNA

Incubation buffer for assay on activated DNA

50 mM Tris-HCl, pH 8.5 (25°C), 15 mM $(NH_4)_2SO_4$, 7 mM $MgCl_2$, 10 mM 2-Mercaptoethanol, 200 µg /ml BSA, 0.1 mM of each dATP, dCTP, dGTP, dTTP.

Incubation procedure

12.5 µg activated calf thymus DNA and 0.1 mCi [α-^{32}P]dCTP are incubated with 0.01 - 0.1 U Tgo DNA polymerase in 50 µl incubation buffer with a paraffin-oil overlay at 72°C for 30 min. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation followed by scintillation counting.

Unit definition

One unit Tgo DNA polymerase is defined as the amount of enzyme that catalyzes the incorporation of 10 nmol total deoxynucleoside triphosphates into acid precipitable DNA within 30 min at 72°C under the conditions described above.

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